$=======$ **ARTICLES** $=======$

Adsorption-Bonded Azo Reagents in Chemical Tests Based on the Principles of Precipitation Paper Chromatography

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Abstract—The possibility of using three groups of azo compounds in chemical tests based on the principles of precipitation paper chromatography is considered. The first group consists of the reagents showing the highest affinity to cellulose (Congo Red, Brilliant Yellow, Stilbazo, Cadion IREA, Sulfarsazen, and Titan Yellow). These reagents are immobilized on paper and are not washed out of it in acidic, alkaline, and neutral solutions. The immobilization of the reagents results in a bathochromic shift of their absorption bands with respect to their solutions and enhances the acidic properties of the reagents. The second group comprises compounds that are immobilized on paper only in acidic solutions (mono- and bisazo derivatives of chromotropic acid: Arsenazo II, Arsenazo III, Orthanilic K, Sulfochlorophenol S, Chlorophosphonazo III, and Carboxyarsenazo). Their immobilization on paper does not lead to a shift in the absorption spectra and acidic properties. The third group consists of compounds that are immobilized on paper either in acidic or in alkaline solutions (Acid Chrome Dark Blue, Eriochrome Black T, Magneson KhS, and Eriochrome Blue Black R). They are absorbed by cellulose as free acids in acidic solutions and as sodium salts in alkaline solutions. Test procedures have been developed for the determination of acids; alkalis (0.1–100 mM); the total alkalinity of water (0.2–35 mM); the total acidity of water (0.2–20 mM); Cd, Al, and Pb (1–200 mg/L); Al, Th, U, and Zr (0.5–200 mg/L); and the total hardness of water (0.05–40 mM). The length of the colored zone in test strips laminated into a polymer film and contacting the test solution through a narrow edge is the analytical signal. The relative standard deviation does not exceed 30%; the analysis time is 10–15 min.

One of the most important problems of chemical tests using immobilized reagents as indicator components is the formation of reagents that cannot be washed off the substrate when it is in contact with the test solution [1]. Reagents covalently immobilized or directly synthesized on both paper [2–4] and silica [5] are most strongly retained. However, at present, these methods of immobilization are of limited use because of the complexity of synthesis procedures and expensiveness.

A simpler method of reagent immobilization is their adsorption retention on paper [1]. The drawback of indicator papers obtained by adsorption immobilization is the weak retention of the reagent by the cellulose substrate. During the immersion of indicator paper in the test solution, the reagent is partially washed out of the paper. The stability of indicator papers is improved by adding stabilizers to the impregnating solution or by coating the paper with transparent polymers [6], which complicates the production of such papers.

Indicator papers with weakly retained reagents cannot be used in chemical tests based on paper precipitation chromatography [7, 8]. Upon the movement of the test solution along the test strip laminated into a polymer film, the reagent is partially washed out and does not form a distinct zone with the test component. However, in these cases, reagents poorly soluble in water or poorly soluble compounds synthesized directly on the paper perform rather well. The paper is impregnated by reagent solutions in organic solvents, or impregnation is carried out in two stages: first with an aqueous solution of the reagent and then, after drying, with a precipitating agent forming a poorly soluble substance with the first reagent in paper pores [9–13].

It was found that substantive (direct) azo compounds can be used in such test systems [14]; their affinity to cellulose paper in some cases is rather high.

The aim of this study is to estimate the degree of affinity of azo reagents to cellulose papers and select conditions for their immobilization and application in chemical tests based on the principles of paper precipitation chromatography.

EXPERIMENTAL

Reagents of cp and analytical grade were used throughout. Stock solutions (1 mg/mL) of U(VI), Th(IV), Zr(IV), Cd(II), Pb(II), and Al(III) were prepared from weighed portions of $UO_2(CH_3COO)_2$, $Th(NO_3)_4 \cdot 4H_2O$, $ZrOCl_2 \cdot 8H_2O$, $Cd(NO_3)_2 \cdot 4H_2O$, $Pb(NO_3)_2$, and $Al(NO_3)_3 \cdot 9H_2O$. Working solutions were prepared by diluting stock solutions on the day of use. Organic azo reagents (pure or cp grade) with a long

π-conjugation chain that are frequently used in inorganic analysis were employed: Congo Red, Brilliant Yellow, Sulfarsazen, Cadion IREA, Stilbazo, Titan Yellow, Arsenazo II, Arsenazo III, Orthanilic K, Sulfochlorophenol S, Chlorophosphonazo III, Carboxyarsenazo, Acid Chrome Dark Blue, Eriochrome Black T, Magneson KhS, and Eriochrome Blue Black R. All the reagents are water-soluble; they were immobilized by immersing cellulose paper into their 0.1% solutions followed by drying.

To prepare indicator papers, ashless filter paper with a density of 75 g/m^2 and an absorbency of 70 mm/10 min was used. An indicator paper strip of size 4 × 80 mm was laminated into a hot adhesive film at a temperature of 100–150°C. PNL-5 films (TU 6-49-57-61-783-334-90) from the Vladimir Chemical Plant were used. The length of the colored zone on the strip was measured using a millimeter-scale ruler (GOST 17435-72). Truncated cones for the test device were prepared from low-pressure polyethylene or polypropylene. To pump a certain volume of the test solution through the test device, a disposable syringe with a rubber piston cup was used. Methods for the production and application of test strips and test devices are described in [8].

The solution acidity was adjusted using acetate–ammonia buffer solutions; the pH was measured with an I-130 potentiometer equipped with a glass electrode and silver–silver chloride electrode. The absorption spectra of indicator papers were measured using an SF-46 or Specol-210 spectrophotometer against paper without an immobilizing agent. The pK values of the reagents in solution and on paper were determined by spectrophotometry [15]. The paper absorbance was measured after impregnating it with a solution with certain acidity and placing it between two thin glass plates. The acidity constants were calculated by the equation

$$K_a = (A_{mx} - A_{HR})[H^+]/(A_R - A_{mx}),$$

where A_{mx}, A_{HR}, and A_R are the absorbances of the mixed, acidic, and dissociated reagent species, respectively, at selected wavelengths.
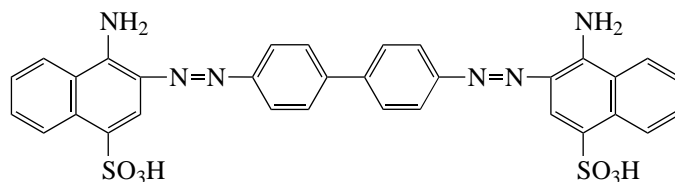
The retention of the azo compounds on paper was estimated by measuring the absorbance of indicator papers after pumping 20 mL of a solution with a certain acidity through the strip using a test device [8]. The retention factor was calculated as: R (%) = $100A/A_0$, where A is the absorbance of the indicator paper after pumping the solution and A_0 is the absorbance of the indicator paper wetted with the solution with a certain acidity. Absorbance was measured against a paper containing no immobilized reagent.
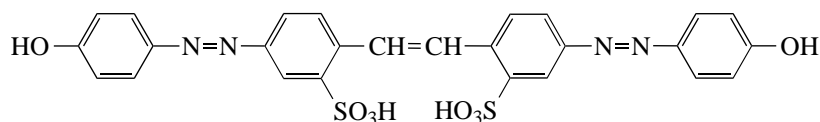
RESULTS AND DISCUSSION

It was found that the selected azo compounds show various degrees of affinity to cellulose paper under different conditions. Therefore, we divided them into three reagent groups: (1) reagents that are retained on paper regardless of the solution acidity; (2) reagents that are retained on paper in acidic solutions; and (3) reagents that are retained on paper in both acidic and alkaline solutions.

Reagents that are retained on paper in acidic, alkaline, and neutral solutions. We assign azo compounds whose degree of affinity to cellulose paper does not depend on the solution acidity to this group. Being immobilized on paper, such reagents are washed out of it neither in acidic nor in alkaline or neutral solutions. The following compounds drop into this group:
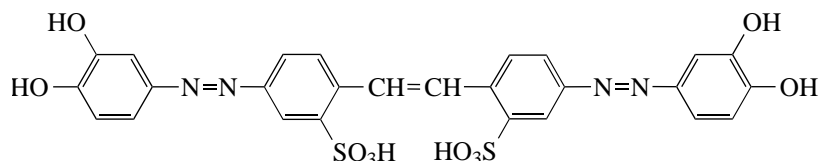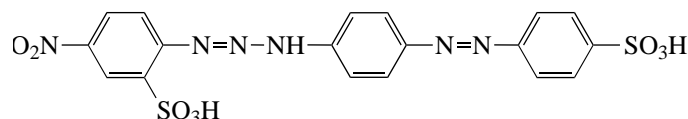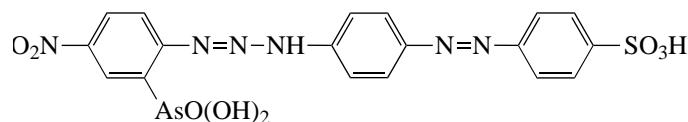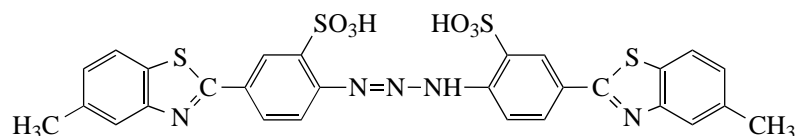
Congo Red

Brilliant Yellow

Stilbazo

Cadion IREA

$$O_2N-\bigcirc-N=N-NH-\bigcirc-N=N-\bigcirc-SO_3H$$
$$SO_3H$$

Sulfarsazen

$$O_2N-\bigcirc-N=N-NH-\bigcirc-N=N-\bigcirc-SO_3H$$
$$AsO(OH)_2$$

Titan Yellow

$$SO_3H \quad HO_3S$$
$$H_3C-\bigcirc\bigcirc_{N}^{S}C-\bigcirc-N=N-NH-\bigcirc-C_{N}^{S}\bigcirc\bigcirc-CH_3$$
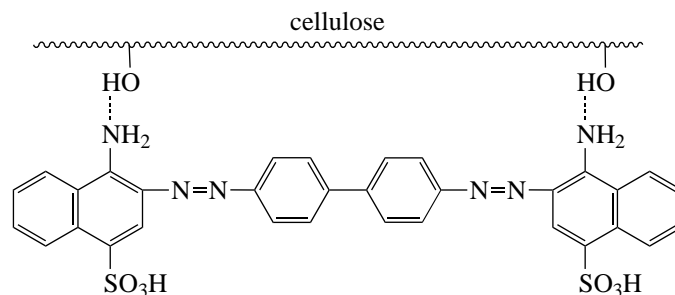
The degree of retention of these reagents is almost independent of the solution acidity and equals 90–98% within the range from 4M HCl to pH 14 (Fig. 1).

These reagents are relatively strongly absorbed by cellulose fibers from aqueous solutions, which is due to specific features of their molecular structures. They are planar, have a long conjugation chain and a minimum number of sulfonic and arsonic groups, and contain groups that can participate in donor–acceptor interactions (OH–, NH_2–, or –N=N–). The linearity and planarity of the molecules and the occurrence of conjugated double bonds serve to enhance the interaction between the reagent and the cellulose macromolecule, which also has a linear structure and a planar arrangement of its fragments:

cellulose

$$HO \quad\quad\quad\quad\quad\quad\quad\quad HO$$
$$NH_2 \quad\quad\quad\quad\quad\quad\quad\quad NH_2$$
$$\bigcirc\bigcirc-N=N-\bigcirc-\bigcirc-N=N-\bigcirc\bigcirc$$
$$SO_3H \quad\quad\quad\quad\quad\quad\quad\quad SO_3H$$

The absorption spectra of reagents immobilized on paper show bathochromic shifts of absorption bands by 10–45 nm compared to the absorption spectra in aqueous solutions (Table 1 and Fig. 2). A decrease in the pKs of reagents by 0.3–0.5 units is also observed. It was found that an increase in the degree of affinity of reagents of this group to cellulose leads to a stronger bathochromic shift of the absorption bands and a decrease in reagent pK (Table 1). This effect can probably be accounted for by the fact that the reagents are retained on cellulose fibers mainly through hydrogen bonds or dipole–dipole interactions rather than through van der Waals forces. Table 1 shows that the strongest effect is observed for Congo Red.

Reagents of this group were used in test systems for determining acids and bases; the total acidity and total alkalinity of water; and cadmium, lead, and aluminum ions (Table 2).

Determination of acids, bases, and the total alkalinity or total acidity of water. Congo Red was used as a reagent (Table 2). In the case of the determination of an alkali or the total alkalinity of water, in addition to Congo Red, either citric acid or $KHSO_4$ were added to impregnating solutions. When the test solution ascends the test strip, alkalis or alkaline components of water react with citric acid (or with $KHSO_4$), the solution pH changes, and a red zone appears in the strip whose length is proportional to the alkali concentration. Ten-

fold amounts of alkali and alkaline-earth metal ions, Al, Fe(III), Zn, Cd, Cu, Ni, Cl$^-$, F$^-$, SO$_4^{2-}$, and PO$_4^{3-}$ and twofold amounts of Hg(II) do not interfere with the determination total alkalinity of 2 mM.

In the case of the determination of acids or the total acidity of water, sodium tetraborate is added to impregnating solutions, and a blue zone appears on the strip, whose length is proportional to the concentration of acids in the solution. Alkali and alkaline-earth metal ions, Al, Fe(III), Zn, Cd, Cu, Ni, Cl$^-$, F$^-$, SO$_4^{2-}$, and PO$_4^{3-}$ (100-fold amounts) and Hg(II) (tenfold amounts) do not interfere with the determination of 5 mM acids.

Table 3 summarizes the results of determining acids, bases, and the total alkalinity and total acidity of water. The relative standard deviation of the analysis results does not exceed 0.3. The analysis time is 15–20 min.

Determination of lead and cadmium. Cadion IREA and Sulfarsazen are used as reagents. In the presence of Pb(II) or Cd(II), a pink zone appears on the yellow strip, whose length is proportional to the concentration in the range 1–200 mg/L (the length of the colored zone is 1–60 mm). The reagents immobilized on paper are not selective, and Zn(II), Cu(II), Ni(II), Co(II), Mn(II), Hg(II), and Ag(I) interfere with the determination; therefore, the reagents can be used for the determination of the test metals after separating or masking the interfering metals.

Determination of aluminum. The known organic reagents for aluminum like Chromazurol S, Eriochromecyanine R, Aluminon, etc., cannot be used in chemical tests based on the principles of precipitation paper chromatography, because they are readily washed out of the paper with the test solution. To determine aluminum we propose Stilbazo. In the presence of aluminum, a pink zone appears on the yellow strip in the range of concentrations 1–200 mg/L. The formed zones are insufficiently contrast; therefore, cetylpyridinium chloride was added to impregnating solutions. It forms ion pairs with the dissociated hydroxy groups of Stilbazo and enhances the degree of contrast of the
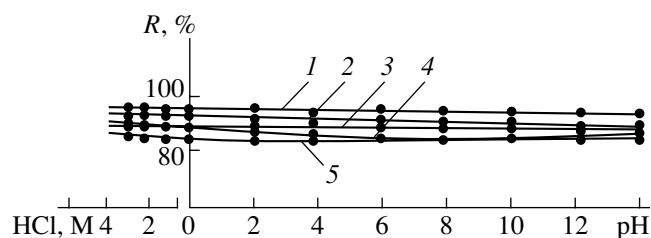

Fig. 1. Dependence of the retention of (*1*) Congo Red, (*2*) Brilliant Yellow, (*3*) Stilbazo, (*4*) Cadion IREA, and (*5*) Sulfarsazen immobilized on paper on the acidity of the pumped solutions. λ, nm: 610 and 520 (Congo Red), 430 and 530 (Brilliant Yellow), 425 and 580 (Cadion IREA), 480 and 420 (Sulfarsazen), and 430 and 540 (Stilbazo).
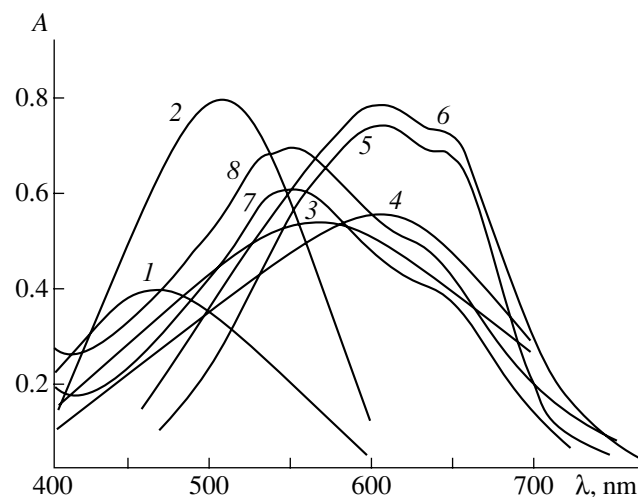

Fig. 2. Absorbance spectra of (*1–4*) Congo Red, (*5, 6*) Eriochrome Black T, and (*7, 8*) Arsenazo III in solution (*1, 3, 5,* and *7*) and immobilized on paper (*2, 4, 6,* and *8*); pH: 1.0 for *3, 4, 7,* and *8*; 10.0 for *1, 2, 5,* and *6*.

reaction (a red zone appears on the yellow strip). The reaction proceeds in more acidic solutions (pH 5–6) in the presence of cetylpyridinium, whereas it proceeds at pH 6–7 without this reagent. Alkali and alkaline-earth metals, Zn(II), Cd(II), NO$_3^-$, NO$_2^-$, Cl$^-$, SO$_4^{2-}$, Fe(II) (1000-fold amounts) and Cu(II), Ni(II), and Co(II)

Table 1. Spectrophotometric parameters of reagents immobilized on paper in acidic, neutral, and alkaline solutions and their pK

| Reagent | λ_{max}, nm | | | λ_{max}, nm | | | pK_a | | |
| | pH < 1 | | | pH > 12 | | | ($I = 0.1$, $n = 5$, $P = 0.95$) | | |
	solution	paper	$\Delta\lambda$, nm	solution	paper	$\Delta\lambda$, nm	solution	paper	ΔpK
Congo Red	570	610	40	480	520	40	3.2 ± 0.2	2.7 ± 0.3	0.5
Brilliant Yellow	405	430	25	485	530	45	8.6 ± 0.1	8.2 ± 0.2	0.4
Cadion IREA	410	425	15	555	580	25	10.9 ± 0.1	10.6 ± 0.2	0.3
Sulfarsazen	465	480	15	410	420	10	2.5 ± 0.2	2.2 ± 0.3	0.3
Stilbazo	400	430	30	500	540	40	7.7 ± 0.2	7.2 ± 0.3	0.5

Table 2. Parameters of test systems with azo reagents immobilized on paper by absorption

Test component	Reagent	Concentration range	Color changes: initial color/zone color	pH_{opt}
Acids	Congo Red	0.1–100 mM	Red/Blue	–
Alkalis	Congo Red + $KHSO_4$	0.1–100 mM	Blue/Red	–
Total alkalinity of water	Congo Red + $KHSO_4$	0.2–35 mM	Magenta/Red	–
Total acidity of water	Congo Red + citric acid	0.2–20 mM	Violet/Blue	–
Cd(II)	Cadion IREA	1–200 mg/L	Yellow/Pink	9–10
Pb(II)	Sulfarsazen	1–200 mg/L	Yellow/Pink	9–10
Al(III)	Stilbazo	1–200 mg/L	Yellow/Pink	6–7
Al(III)	Stilbazo + Cetylpyridinium	0.5–200 mg/L	Yellow/Red	5–6
Th(IV)	Arsenazo III	0.5–200 mg/L	Red/Green	2–3 M HCl
U(VI)	Arsenazo III	0.5–200 mg/L	Red/Blue	2–3 M HCl
Zr(IV)	Arsenazo III	0.5–200 mg/L	Red/Blue	2–3 M HCl
Total hardness of water	Eriochrome Black T + $Na_2B_4O_7$	0.05–40 mM	Green/Red	9–12

Table 3. Results of determining acids, bases, the total alkalinity and total acidity of water, and some ions ($n = 3$; $P = 0.95$)

Test sample	Parameter (component) to be determined	Found by chemical tests, mM (mg/L)		Found by titrimetry, mM (added, mg/L)
		$x \pm \Delta$	RSD, %	
Solution	Acid (HCl)	5 ± 2	16	5.85 ± 0.06
		10.2 ± 0.9	4	10.83 ± 0.08
Solution	Alkali (NaOH)	3.5 ± 0.6	7	3.85 ± 0.07
		45 ± 2	2	46.3 ± 0.4
Tap water	Total alkalinity	2.3 ± 0.8	14	2.25 ± 0.06
		7.5 ± 0.6	3	8.00 ± 0.02
Tap water	Total acidity	1.3 ± 0.6	18	1.45 ± 0.08
		3.2 ± 0.8	1	3.45 ± 0.06
Model solutions	Al(III)	(1.8 ± 0.8)	18	(2.0)
		(3 ± 1)	13	(3.0)
the same	Cd(II)	(5 ± 1)	8	(6.0)
"	Pb(II)	(10 ± 2)	8	(10.0)
"	Th(IV)	(6.2 ± 0.8)	5	(6.0)
"	U(VI)	(15.3 ± 0.9)	2	(14.0)
"	Zr(IV)	(12 ± 2)	7	(13.0)

(100-fold amounts) do not interfere with the determination of aluminum. Equal amounts of Fe(III), F^-, and PO_4^{3-} interfere. The interference from 50-fold amounts of Fe(III) was eliminated by adding ascorbic acid.

The optimum pH value of the test solution was adjusted with acetate and borate buffer solutions. A paper impregnated with a 0.1% solution of sodium acetate, cetylpyridinium (in the determination of Al(III)), sodium tetraborate (in the determination of Cd(II) and

Pb(II)) was used. A square strip of such paper (1×1 cm) was immersed in 1 mL of the sample.

Table 3 shows the results of determining Al(III), Cd(II), and Pb(II) in model solutions (tap water). The composition of the model solutions was as follows: pH 7.2; total hardness of water, 6 mM; total alkalinity, 2 mM; active chlorine, 0.4 mg/L; chlorides, 10 mg/L; nitrates, 6 mg/L; NH_4^+, 0.5 mg/L; Cu, 0.01 mg/L; sulfates, 80 mg/L; phosphates, 2 mg/L; fluorides, 0.3 mg/L, Zn, 0.5 mg/L; and iron, 0.2 mg/L. Table 3 shows that the components of a model solution do not significantly affect the accuracy of the determination. The relative standard deviation does not exceed 0.3. The analysis time is 10–15 min.

Reagents retained on paper in acidic solutions. Mono- and bis-azo derivatives of chromotropic acid fall into this group:
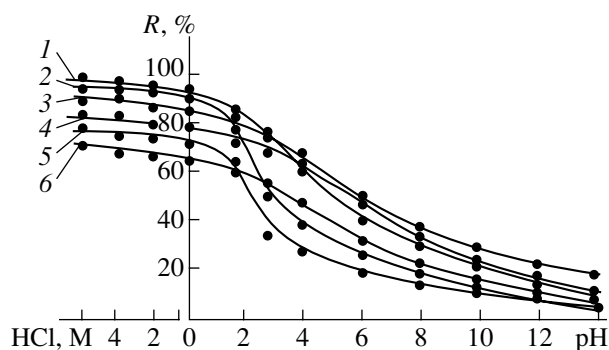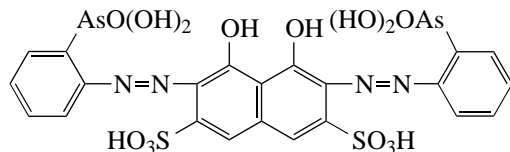

Fig. 3. Dependence of the retention of (*1*) Arsenazo III, (*2*) Arsenazo II, (*3*) Orthanilic K, (*4*) Carboxyarsenazo, (*5*) Sulfochlorophenol S, and (*6*) Chlorophosphonazo III immobilized on paper on the acidity of pumped solutions (540 and 600 nm).
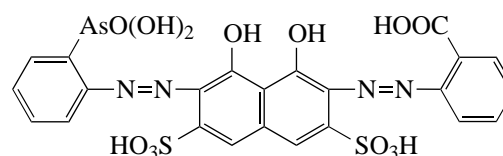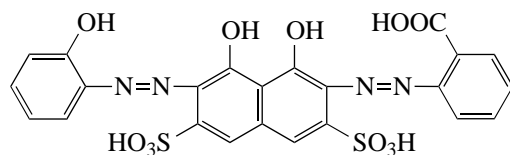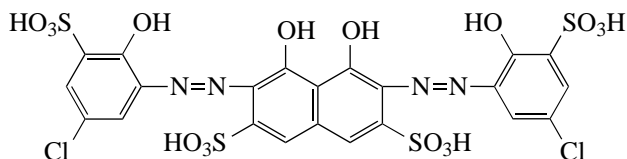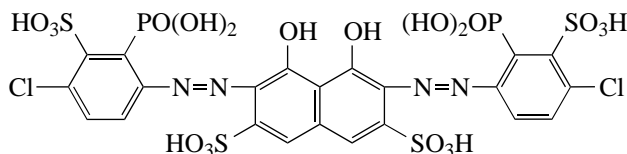
Arsenazo II
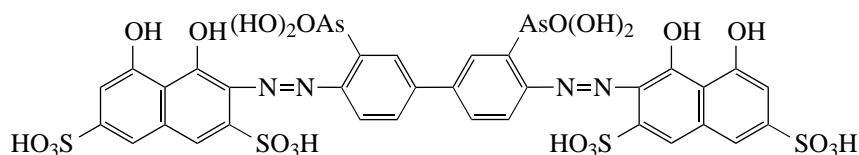
Arsenazo III

Orthanilic K

Sulfochlorophenol S

Chlorophosphonazo III

Carboxyarsenazo

As distinct from the reagents of the first group, these reagents have a larger number of sulfonic, arsonic, or phosphonic groups. The reagents are washed out of paper at pH > 2 and are not washed out in highly acidic solutions (the degree of retention is 80–90% in 0.5–6 M HCl, Fig. 3). Probably, under these conditions the dissociation of these groups is suppressed, which leads to a decrease in their solubility. Moreover, the reagents are protonated, so the negatively charged molecules are partially neutralized and the dipole–dipole interaction with the negatively charged surface of cellulose fibers become stronger. The absorbance spectra of the reagents immobilized on paper and in solution are identical (Fig. 1). The pK values of the reagents both on paper and in solution also do not change.

Test systems for the determination of thorium(IV), uranium(VI), and zirconium(IV) with Arsenazo III in acidic solutions are proposed. This reagent was selected because it forms the most contrast and distinct colored zones on test strips with these elements.

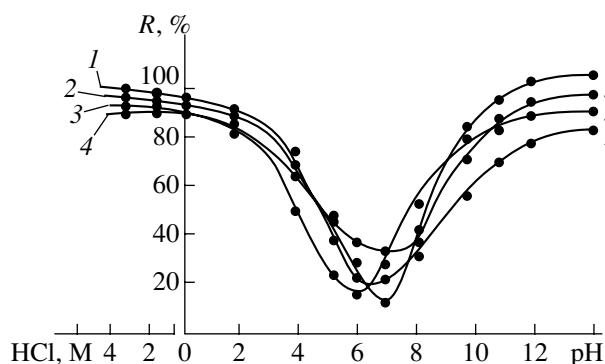**Determination of thorium, uranium, and zirconium.** In the presence of these elements, a green or

Fig. 4. Dependence of the degree of retention of (*1*) Eriochrome Black T, (*2*) Acid Chrome Dark Blue, (*3*) Eriochrome Blue Black R, and (*4*) Magneson KhS immobilized on paper on the acidity of pumped solutions (500, 440, and 620 nm).

blue zone appears on the red test strip in the concentration range 0.5–200 mg/L (the colored-zone length is 1−60 mm). The optimum acidity is 2–3 M HCl. The test elements affect the determination of each other. In the determination of thorium, the interference from 100-fold amounts of zirconium can be eliminated using oxalic acid; in the determination of uranium, tenfold amounts of thorium and zirconium were masked by adding fluorides. Alkali and alkaline-earth metals, Al(III), Pb(II), Zn(II), Cd(II), Ni(II), Fe(III), Cu(II), SO_4^{2-}, and PO_4^{3-} (1000-fold amounts) and 100-fold amounts of fluorides do not interfere with the determination.

The results of determining these elements in model solutions are presented in Table 3. The relative standard deviation does not exceed 0.3; the analysis time is 10–15 min.

Reagents retained on paper in both acidic and alkaline solutions. This group comprises monoazo compounds bearing one or two sulfonic groups and producing their sodium salts
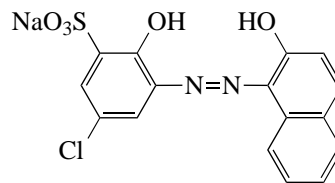
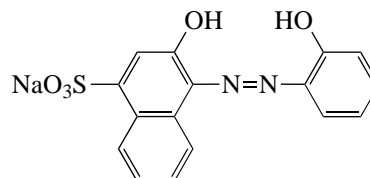Acid Chrome Dark Blue
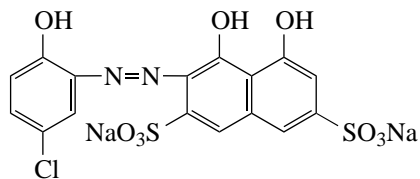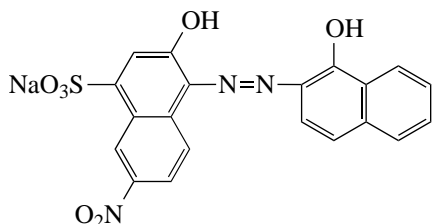
Eriochrome Black T

Magneson KhS

Eriochrome Blue Black R

Being immobilized on paper, the reagents of this group are washed out at pH 3–6 and are not washed out in alkaline (pH 9–14) or acidic (0.01–4 M HCl) solutions. Eriochrome Black T is most strongly retained; its degree of retention is 90–95% (Fig. 4). These compounds belong to the group of acidic dyes and are absorbed by fibers as free acids from acidic solutions and as sodium salts from alkaline solutions. As for the substances of the second group, the absorbance spectra of the reagents on paper and in solution do not show any changes, and the p*K*s of the reagents also do not change (Fig. 2).

As an example of the application of the reagents of this group, we present here test procedures for the determination of the total hardness of water using Eriochrome Black T.

Determination of the total hardness of water. Total hardness can be determined either by the length of the colored zone of the test strip or by the color intensity of indicator papers after pumping a certain volume of the test liquid through the strip. In all cases, the paper is impregnated with aqueous solutions of Eriochrome Black T, sodium tetraborate, and a magnesium chelate. The latter substance is added to make the change in the color of the reagents more distinct in the presence of calcium ions. Alkaline-earth metal ions change the color of the paper from blue to magenta.

It was found that the complexes of calcium and magnesium formed with Eriochrome Black T are strongly absorbed by cellulose paper and are not washed out after pumping up to 20 mL of water through the paper using a test device. The calibration ranges of total concentrations of alkaline-earth metal ions are 0.05–40 mM using test strips (the length of the colored zone is 1–70 mm) and 0.001–0.1 mM using the test device (the volume of the pumped solution is 20 mL).

Heavy-metal ions at concentrations of ≥0.1 mg/L interfere with the determination of alkaline-earth metal ions. To eliminate their effect, sodium sulfide or diethyldithiocarbaminate should be added to the test sample. Under field conditions, it is convenient to use dried indicator paper (1 × 1 cm in size) impregnated with a

Table 4. Results of determining the total hardness of water ($n = 5$; $P = 0.95$)

Test sample	Found by chemical tests, mM		Found by titrimetry, mM
	test strips (RSD)	test devices (RSD)	
Water of the Klyaz'ma River	5.4 ± 0.8 (0.13)	–	5.8 ± 0.04
Water of the Nerl' River	4.8 ± 0.6 (0.10)	–	5.02 ± 0.03
Tap water	6.0 ± 0.5 (0.07)	–	5.92 ± 0.03
Spring water	12 ± 2 (0.13)	–	11.8 ± 0.4
Well water	7.2 ± 0.6 (0.07)	–	7.45 ± 0.05
Distilled water	0.05 ± 0.02 (0.32)	0.05 ± 0.01 (0.16)	0.044 ± 0.003
Deionized water	–	0.008 ± 0.002 (0.21)	0.007 ± 0.001
Feedwater of steam boilers No. 1	–	0.03 ± 0.01 (0.27)	0.023 ± 0.006
No. 2	25 ± 3 (0.10)	–	25.5 ± 0.2
Turbine condensate	–	0.006 ± 0.002 (0.27)	0.007 ± 0.002

0.1% solution of sodium diethyldithiocarbaminate. A single piece of this paper is introduced in 20 mL of the test liquid, and up to 10 mg/L of Zn, Pb(II), Fe(III), Ni, Mn(II), Cu(II), Co, Cd, and Hg(II) are masked.

Sodium tetraborate added to the impregnating solution provides the optimum pH 10–11 for the determination of alkaline-earth metals, while the test solution may have pH 6–11.

Test strips were used for determining the total hardness of natural, tap, and process waters. The analysis time is 10–15 min, and the relative standard deviation does not exceed 0.3 (Table 4). To determine the hardness of distilled or cationized water, a sample of 20 mL was pumped through a test device with an indicator paper for 3–5 min. The color intensity of the reaction zone of the paper was estimated using a standard colorimetric scale; in this case, the relative standard deviation of the analysis results is 0.1–0.3 (Table 3).

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